Clin Drug Invest. 9 (1): 43-53, 1995
0114-2402/95/0001-0043/$05.50/0



# Pharmacokinetics of Sulfamethoxazole with its Hydroxy Metabolites and N4-Acetyl-, N1-Glucuronide Conjugates in Healthy Human Volunteers

*Tom B. Vree,*[1,2] *André J.A.M. van der Ven,*[3] *Peter P. Koopmans,*[3] *Eleonora W.J. van Ewijk-Beneken Kolmer*[1] and *Corrien P.W.G.M. Verwey-van Wissen*[1]

1 Department of Clinical Pharmacy, Academic Hospital Nijmegen Sint Radboud, Nijmegen, The Netherlands
2 Institute of Anaesthesiology, Academic Hospital Nijmegen Sint Radboud, Nijmegen, The Netherlands
3 Department of Internal Medicine, Division of General Internal Medicine, Academic Hospital Nijmegen Sint Radboud, Nijmegen, The Netherlands

## Summary

The aim of this investigation was to assess the pharmacokinetics of sulfamethoxazole (S) with its hydroxy metabolites (SOH, $N_4$SOH, $N_4$OH) and $N_4$-acetyl- ($N_4$) and $N_1$-glucuronide (Sgluc) conjugates in 7 human volunteers after an oral dose of 800mg using a reversed phase gradient high-pressure liquid chromatography (HPLC) with UV detection. Sulfamethoxazole was rapidly and completely absorbed and metabolised to 5 metabolites. The plasma half-life ($t_{½}$) of elimination varied for the parent drug and its metabolites between 9.7 and 15 hours. The protein binding of S (67.2%) increased when the compound was acetylated (88%), and decreased when it was oxidised at the 5-position (40%). Glucuronidation at the $N_1$-position reduced the protein binding to 20%. The main metabolite in urine was $N_4$ (43.5 ± 5.6%), followed by S (14.4 ± 3.4%). The percentages of the Sgluc (9.8 ± 2.6%), $N_4$SOH (5.3 ± 1.0%), and SOH (3.0 ± 1.0%) did not differ statistically (p = NS). Only 2 to 3% of the N-hydroxylamine metabolite ($N_4$OH) was excreted. The renal clearance values were: Sgluc 176 ± 33 ml/min, SOH 96.1 ± 23.7 ml/min, $N_4$SOH 51.2 ± 10.4 ml/min, $N_4$ 35.2 ± 5.6 ml/min and S 2.7 ± 0.9 ml/min. The pharmacokinetic behaviour of the $N_1$-glucuronide was reported for the first time. If one of the metabolites is responsible for the occurrence of side effects, then all metabolites must be included in this analysis.

The known metabolism of sulfamethoxazole involves acetylation and oxidation at the $N_4$ nitrogen atom leading to $N_4$-acetylsulfamethoxazole ($N_4$) and $N_4$-hydroxysulfamethoxazole ($N_4$OH).[1-4] The latter compound is thought to be responsible for the occurrence of adverse effects with cotrimoxazole treatment for *Pneumocystis carinii* pneumonia in HIV-positive patients.[5] Hydroxylation also takes place at the $C_5$ methyl group, leading to 5-hydroxysulfamethoxazole (SOH) and $N_4$-

acetyl-5-hydroxysulfamethoxazole ($N_4$SOH).[3,6-10] The existence of sulfamethoxazole-$N_1$-glucuronide (Sgluc) in urine has been described.[3,6-10] The molecular structures of sulfamethoxazole and its metabolites are shown in figure 1.

Sulfamethoxazole is predominantly eliminated by $N_4$-acetylation, the metabolite being eliminated by active tubular secretion.[3,11-14] The renal elimination of sulfamethoxazole occurs via the passive renal processes of glomerular filtration minus passive tubular reabsorption, implying that the renal clearance depends primarily on protein binding and urine pH, and to a minor extent on urine flow. With an acidic urine pH the renal clearance of sulfamethoxazole is low (2.5 ml/min), and with an alkaline urine pH it is high (25 ml/min).[11-14]

Recently we investigated the pharmacokinetics of the N-hydroxylamine of sulfamethoxazole in human volunteers by isocratic as well as gradient high-pressure liquid chromatography (HPLC), and observed the presence of the $N_1$-glucuronide conjugate of sulfamethoxazole.[15]

The pharmacokinetics for $N_1$-glucuronides of sulfonamides have previously been described for sulfadimethoxine,[16,17] sulfa-6-monomethoxine,[18] sulfamethomidine,[19] and sulfaphenazole,[20] but not for sulfamethoxazole. $N_1$-glucuronides of sulfonamides can be measured well by direct gradient HPLC analysis.

The aim of this study was to investigate the pharmacokinetics of sulfamethoxazole with its hydroxy metabolites, and $N_4$-acetyl- and $N_1$-glu-

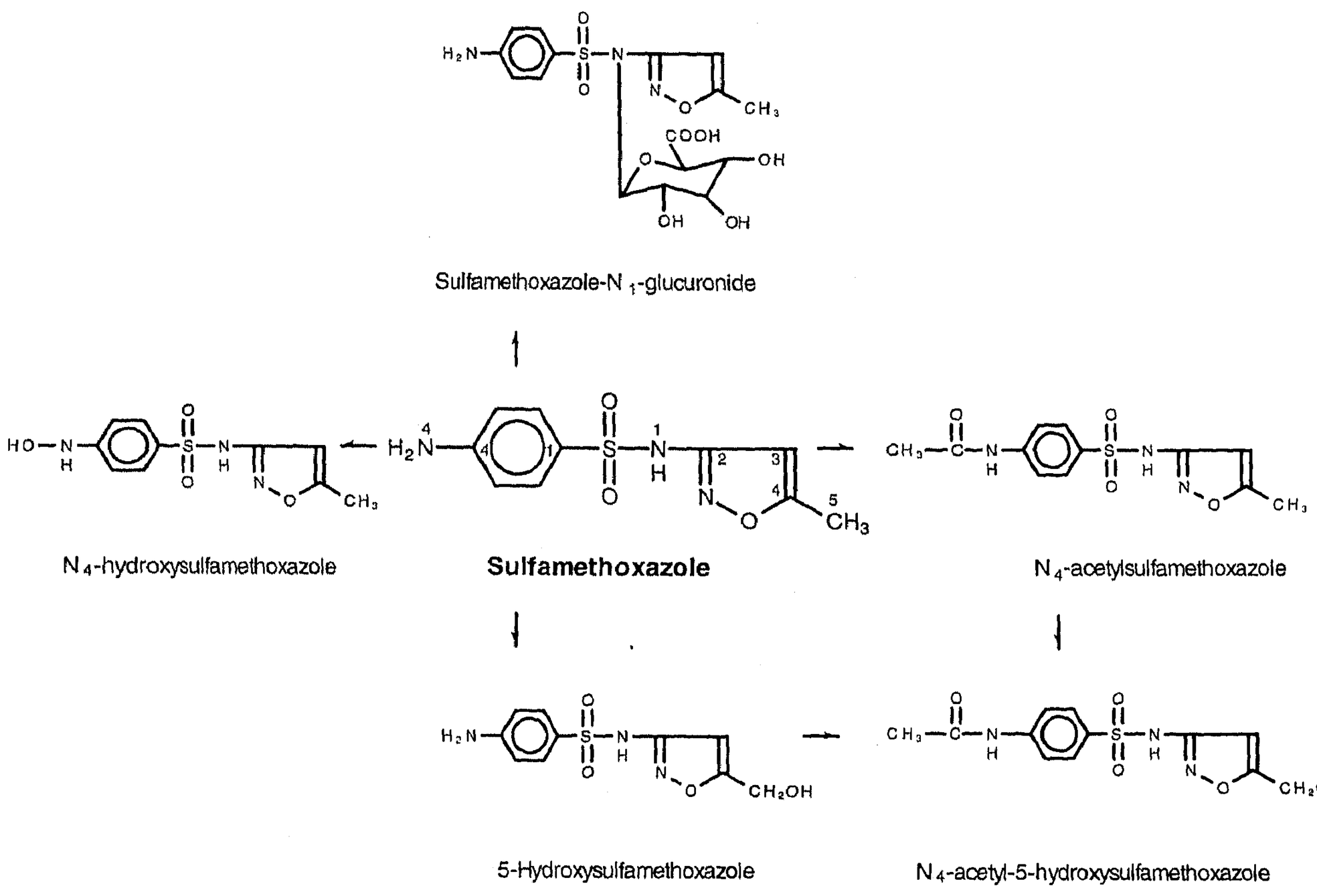


**Fig. 1.** Molecular structures of sulfamethoxazole and its metabolites.

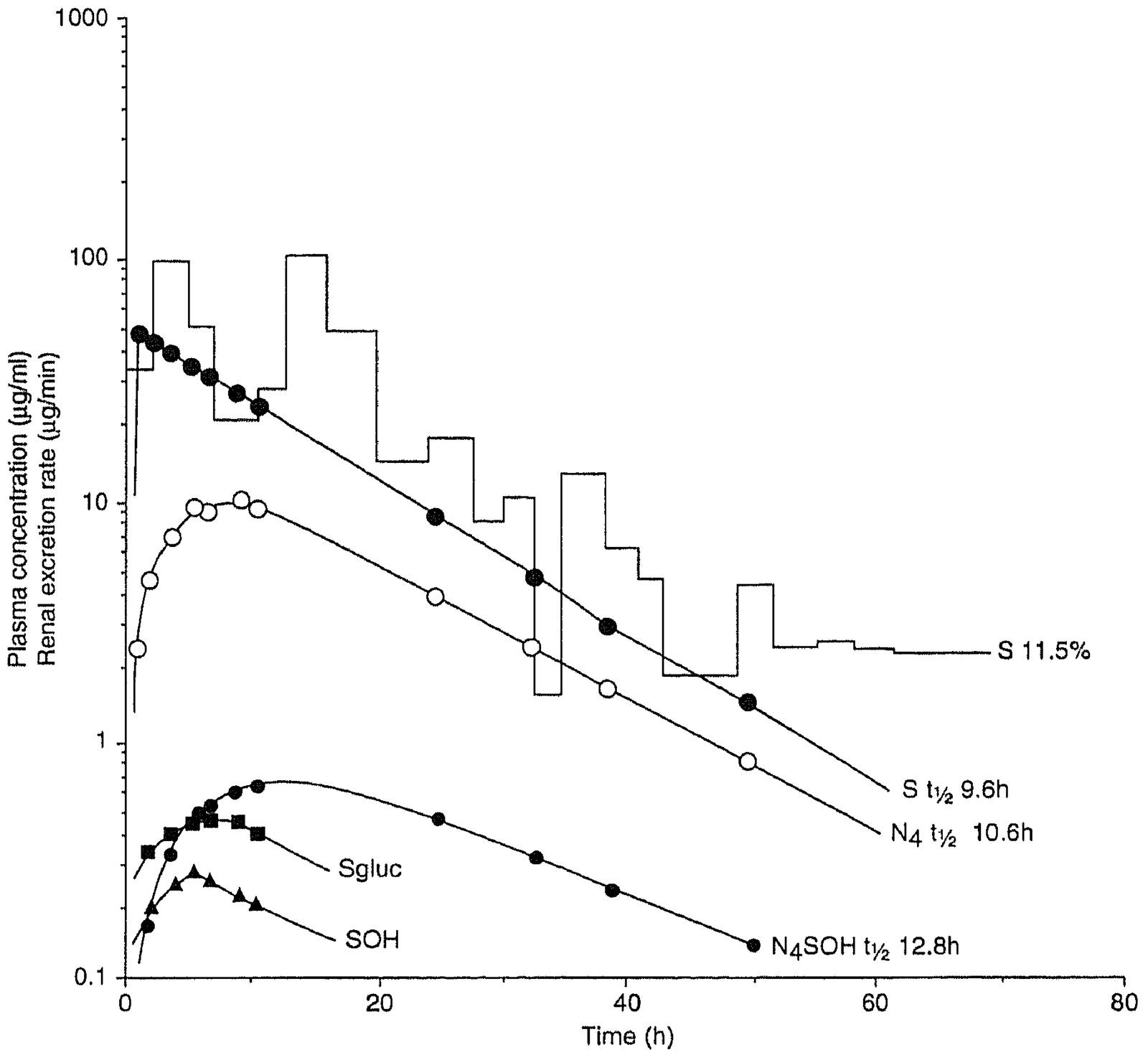


**Fig. 2.** Plasma concentration versus time curves of sulfamethoxazole (S), sulfamethoxazole-$N_1$-glucuronide (Sgluc), 5-hydroxysulfamethoxazole (SOH), $N_4$-acetylsulfamethoxazole ($N_4$) and $N_4$-acetyl-5-hydroxysulfamethoxazole ($N_4$SOH) in a volunteer (no. 3) after a single oral dose of sulfamethoxazole 800mg. For reasons of clarity, only the renal excretion rate-time profile and cumulative amount excreted (% mol dose) of sulfamethoxazole are given.

curonide conjugates in human plasma and urine by direct gradient HPLC analysis.

The pharmacokinetic data obtained can thereafter be compared with those obtained from HIV-positive patients, in order to detect discrepancies in metabolism that may be related to the occurrence of side effects

## Participants and Methods

### Participants

Seven human volunteers (4 males, 3 females; 4 fast acetylators and 3 slow acetylators of sulfadimidine;[21] age 30 ± 10 years; bodyweight 75 ± 8kg) ingested a single oral dose of sulfamethoxazole 800mg in one gelatine capsule (No XX) after an overnight fast.

The study was approved by the hospital ethics committee, and informed consent was obtained from the volunteers.

### Sampling

Blood samples were collected in heparinised Eppendorf vessels (2ml) at regular time intervals over 2 days after administration by means of fingertip puncture with Monolet® lancets (Monoject, St

Louis, USA). After centrifuging at 3000*g*, plasma samples were stored at –20°C pending analysis.

Urine was collected upon untimed voiding. The total time of sample collection was 96 hours. Three samples (7ml) of each void were stored at –20°C pending analysis.

## Drug Analysis

Sulfamethoxazole and all metabolites were analysed by direct HPLC analysis as described in detail elsewhere.[15]

## Sample Treatment

Plasma samples (100µl) were deproteinised with 100µl acetonitrile, centrifuged at 3000*g*, and 20µl of the supernatant was injected onto the column.

### *Urine Samples*

For the measurement of $N_4OH$, urine was centrifuged at 3000*g*, the supernatant was diluted 1:9 with 0.2 mol/L $KH_2PO_4$ buffer pH 5.0, and 20µl was injected onto the column.

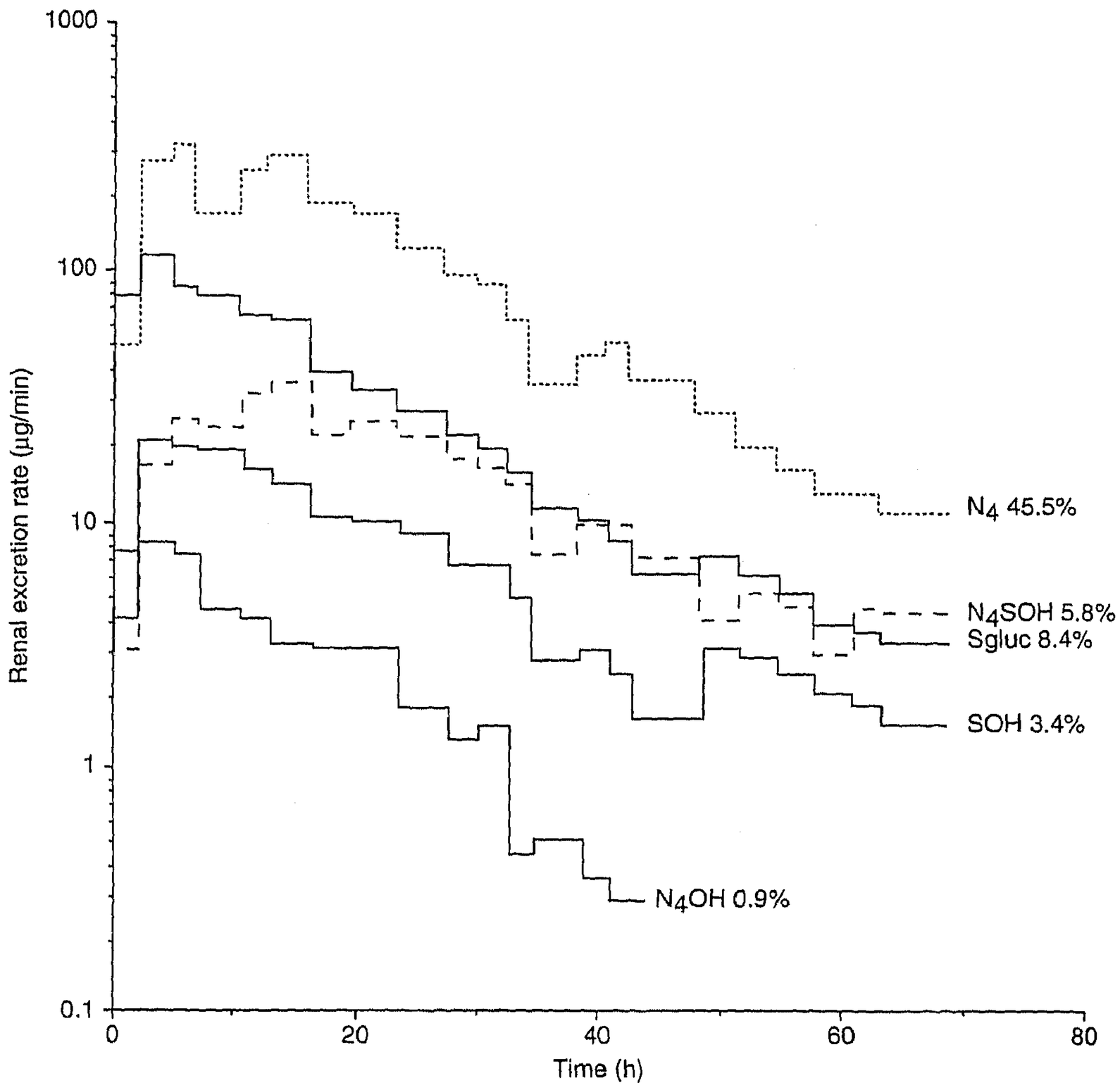


**Fig. 3.** Renal excretion rate-time profiles and cumulative amount excreted (% mol dose) of sulfamethoxazole-$N_1$-glucuronide (Sgluc), 5-hydroxysulfamethoxazole (SOH), $N_4$-acetylsulfamethoxazole ($N_4$), $N_4$-acetyl-5-hydroxysulfamethoxazole ($N_4SOH$), and $N_4$-hydroxysulfamethoxazole ($N_4OH$) in a volunteer (no. 3) after a single oral dose of sulfamethoxazole 800mg. For reasons of clarity, the renal excretion rate-time profile of sulfamethoxazole is omitted in this figure and is shown in figure 2.

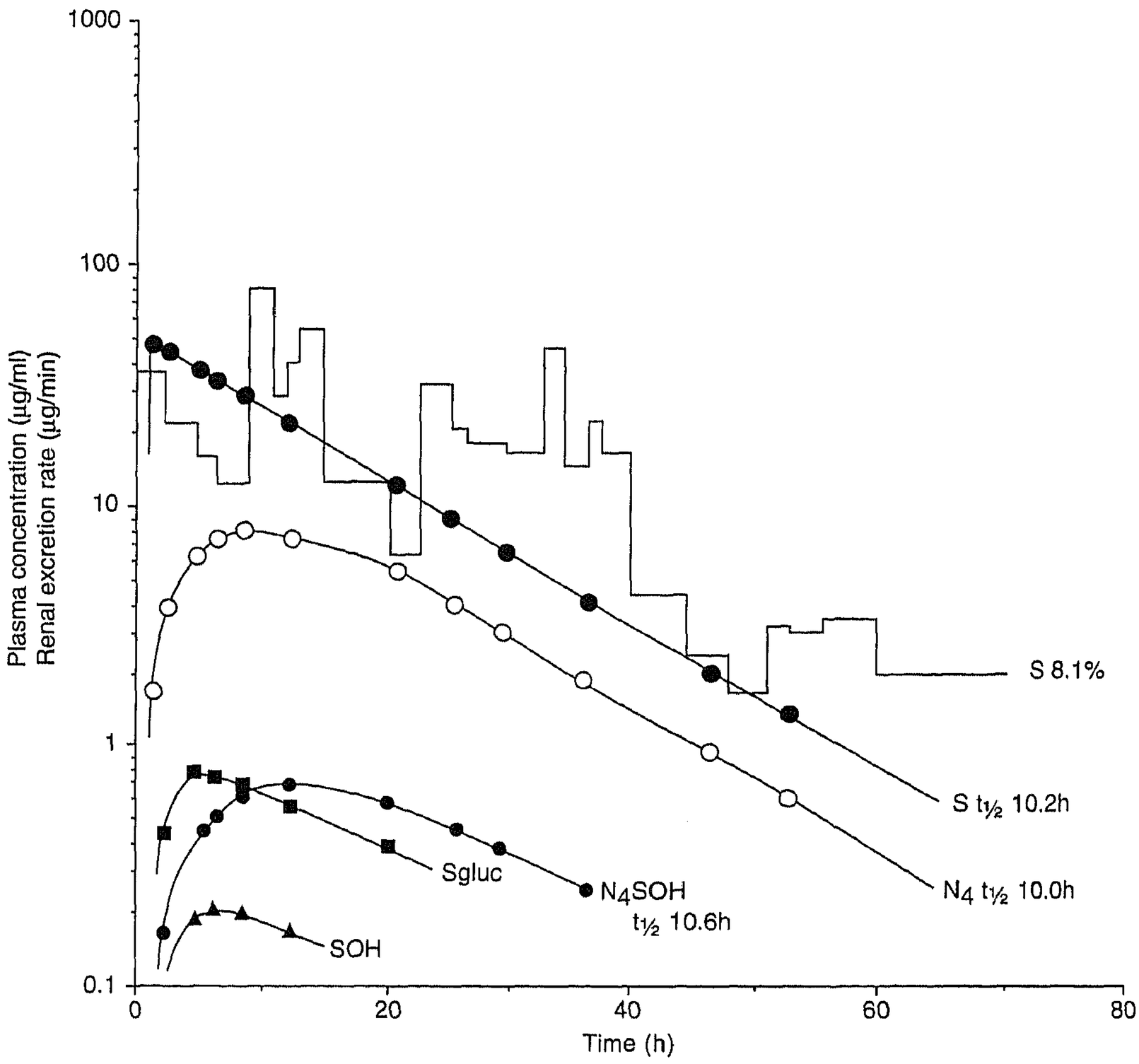


**Fig. 4.** Plasma concentration versus time curves of sulfamethoxazole (S), sulfamethoxazole-$N_1$-glucuronide (Sgluc), 5-hydroxysulfamethoxazole (SOH), $N_4$-acetylsulfamethoxazole ($N_4$) and $N_4$-acetyl-5-hydroxysulfamethoxazole ($N_4$SOH) in a volunteer (no. 4) after a single oral dose of sulfamethoxazole 800mg. For reasons of clarity, only the renal excretion rate-time profile of sulfamethoxazole is given.

For the measurement of sulfamethoxazole and the other metabolites, 50µl diethylamine was added to the urine sample of 7ml (pH 10) to increase the solubility of all sulfonamides, centrifuged at 3000*g*, and thereafter the supernatant was diluted 1:9 with 0.2 mol/L $K_2PO_4$ buffer pH 5.0.

### Intraday-Interday Coefficient of Variation

The intraday and interday coefficients of variation of sulfamethoxazole and its metabolites were <5%.[15]

### Protein Binding

The protein binding (*in vitro*) of sulfamethoxazole and its metabolites was measured in blank plasma samples by means of the Amicon Micropartition system MPS-1 (Grace BV, Amicon Division, Capelle aan de IJssel, The Netherlands). The average protein binding was calculated from 4 spiked-plasma samples, equilibrated for 15 minutes at 37°C. Minimal drug binding to the filters was observed.

**Table I.** Pharmacokinetic parameters of sulfamethoxazole (S) after a single oral dose of 800mg to healthy volunteers (n = 7)

| Parameter | Mean ± SD | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | S | p value[a] | $N_4$ | p value[b] | $N_4SOH$ | p value[c] | Sgluc | p value[d] | SOH |
| $C_{max}$ (mg/L) | 50.2 ± 9.6 | <0.0001 | 8.3 ± 2.0 | 0.0093 | 0.56 ± 0.14 | | 0.77 ± 0.31 | | 0.38 ± 0.17 |
| $t_{lag}$ (h) | 0.29 ± 0.41 | | 0.48 ± 0.49 | | 1.11 ± 0.89 | | 1.21 ± 1.00 | | 0.74 ± 1.03 |
| $t_{max}$ (h) | 1.0 ± 0.8 | <0.0001 | 6.9 ± 1.7 | 0.0019 | 10.4 ± 1.7 | 0.0020 | 5.5 ± 3.4 | | 9.3 ± 3.8 |
| $t_{½\ abs}$ (h) | 0.12 ± 0.14 | 0.0146 | 2.3 ± 0.9 | 0.0163 | 4.5 ± 2.4 | 0.0117 | 1.3 ± 1.4 | | 4.7 ± 5.0 |
| $t_{½}$ (h) | 9.7 ± 1.3 | | 11.2 ± 1.7 | | 11.1 ± 3.3 | 0.0352 | 15.2 ± 5.3 | | 9.5 ± 1.7 |
| MRT (h) | 14.4 ± 2.2 | 0.0044 | 19.8 ± 2.9 | 0.0289 | 24.1 ± 3.1 | | 25.1 ± 6.8 | | 21.5 ± 3.5 |
| $MRT_i$ (h) | [e] | | 5.7 ± 1.9 | | 10.1 ± 3.0 | | 9.6 ± 7.6 | | 20.6 ± 14.2 |
| $MRT_{ij}$ (h) | [e] | | [e] | | 3.9 ± 1.9 | | [e] | | [e] |
| AUC (mg/L•h) | 742. ± 201 | <0.0001 | 198. ± 41 | 0.0052 | 20.3 ± 7.5 | | 19.8 ± 4.9 | | 12.5 ± 5.7 |
| $CL_o$ (L/h) | 0.93 ± 0.24 | 0.0133 | 1.9 ± 0.3 | | 2.5 ± 0.7 | 0.0002 | 4.9 ± 1.7 | | 4.8 ± 2.0 |
| $V_{ss}$ (L) | 12.7 ± 2.5 | 0.0537 | 30.3 ± 7.9 | | 42.9 ± 19.0 | 0.0005 | 102.6 ± 30.4 | | 94.7 ± 53.4 |
| % dose excreted[f] | 14.4 ± 3.4 | <0.0001 | 43.5 ± 5.6 | <0.0001 | 5.3 ± 1.0 | 0.0091 | 9.8 ± 2.6 | 0.0004 | 3.0 ± 1.0 |
| $CL_R$ (ml/min) | 2.7 ± 0.9 | <0.002 | 35.2 ± 5.6 | | 51.2 ± 10.4 | <0.0001 | 176 ± 33 | <0.0001 | 96.1 ± 23.7 |

a Difference between S and $N_4$.

b Difference between $N_4$ and $N_4SOH$.

c Difference between $N_4SOH$ and Sgluc.

d Difference between Sgluc and SOH.

e Not stable in plasma.

f % dose excreted for sulfamethoxazole-N-hydroxylamine = 2.1 ± 0.9%.

*Abbreviations:* AUC = area under the concentration-time curve; $CL_o$ = oral clearance; $CL_R$ = renal clearance; $C_{max}$ = maximum plasma drug concentration; MRT = mean residence time; $MRT_i$ = intrinsic mean residence time (metabolite - parent); $MRT_{ij}$ = intrinsic mean residence time (metabolite j - metabolite i); $N_4$ = $N_4$-acetylsulfamethoxazole; $N_4SOH$ = $N_4$-acetyl-5-hydroxysulfamethoxazole; Sgluc = sulfamethoxazole-$N_1$-glucuronide; SOH = 5-hydroxysulfamethoxazole; $t_{lag}$ = lag time; $t_{max}$ = time to reach maximum concentration; $t_{½}$ = half-life; $t_{½\ abs}$ = absorption half-life; $V_{ss}$ = apparent volume of distribution at steady-state.

### Drugs

Sulfamethoxazole and $N_4$-acetylsulfa-methoxazole were obtained from Hoffmann-LaRoche (Mijdrecht, The Netherlands). The metabolites 5-hydroxysulfamethoxazole, $N_4$-acetyl- 5-hydroxysulfamethoxazole, and sulfamethoxazole- $N_1$-glucuronide were isolated from human urine as described elsewhere.[15] $N_4$-hydroxysulfamethoxazole was synthesised by Synthon (Nijmegen, The Netherlands). All other reagents were of proanalysi quality and obtained from Merck (Darmstadt, Germany).

### Pharmacokinetics

Curve fitting was carried out, and pharmacokinetic parameters were calculated using the MediWare® computer package.[22] The area under the plasma concentration-time curve from zero to infinity ($AUC_{0-\infty}$) values were calculated using the linear trapezoidal rule with extrapolation to infinity from C(last)/$\lambda_z$:

$$MRT = AUMC/AUC = V_{ss}/CL + 1/k_a + t_{lag};$$

$$t_{½z} = \ln 2/\lambda_z$$

where MRT is the mean residence time, AUMC is the area under the moment curve, $V_{ss}$ is the apparent volume of distribution at steady-state, CL is the total body clearance, ka is the absorption rate constant, $t_{lag}$ is the lag time and $t_{½z}$ is the elimination half-life.

$$CL_{o,parent} = F \cdot D/AUC_{0-\infty}$$

where $CL_{o,parent}$ is the oral clearance of the parent drug, and the bioavailability F taken as the total

percentage of parent drug and metabolites of the molar dose (μmol) excreted in the urine.

$$CL_{o,metabolite} = F_m \cdot D/AUC_{0-\infty}$$

where $CL_{o,metabolite}$ is the oral clearance of the metabolite, and the bioavailability of the metabolite $F_m$ is approximately equal to the percentage of the molar dose excreted in the urine as metabolite.

Renal clearance $CL_R$ = mg excreted/$AUC_{0-\infty}$, which corresponds to the average renal clearance obtained from the renal excretion rate divided by the extrapolated plasma concentration at the midpoint of the urine collection period (n = 5). For metabolites with a small $AUC_{0-\infty}$, the average renal clearance was calculated.

$$MRT = MRT_{absorption} + MRT_{disposition}$$

with $MRT_{absorption} << MRT_{disposition}$.

$$MRT_i = MRT_{metabolite} - MRT_{parent}$$

where $MRT_i$ is the intrinsic mean residence time, and

$$MRT_{ij} = MRT_{metabolite\ ij} - MRT_{metabolite\ i}(N_4SOH\text{-}N_4).$$

### Statistical Analysis

Regression lines, standard deviations, and analysis of variance (ANOVA) were calculated according to standard statistical procedures.[23] Statistical significance was defined as $p<0.05$.

## Results

Figure 2 shows an example of plasma concentration-time curves and figure 3 the corresponding renal excretion rate-time profiles of sulfamethoxazole and its metabolites in one male volunteer (no. 3). In this particular volunteer, the $t_{1/2}$ of the metabolites in plasma were slightly higher than that of the parent drug. Figure 4 shows identical half-lives of the parent drug and metabolites in the plasma concentration-time curves of sulfamethoxazole in another volunteer (no. 4).

Table I summarises the mean ± SD values of the measured pharmacokinetic parameters of sulfamethoxazole in the volunteers.

The lag time indicates the length of time elapsed before the appearance of a measurable plasma concentration of drug and metabolites. The average sequence of appearance in the blood was sulfamethoxazole 0.29 ± 0.41 hours, $N_4$ at 0.48 ± 0.49 hours (p = NS), SOH at 0.74 ± 1.03 hours (p = NS), $N_4$SOH at 1.11 ± 0.89 hours (p = 0.028), finally followed by Sgluc at 1.21 ± 1.00 hour (p = 0.049; p values compared with sulfamethoxazole.

The time at which the maximum concentration ($C_{max}$) was reached ($t_{max}$) was the shortest for the parent drug (1.02 ± 0.84 hours), followed by that for Sgluc at 5.5 ± 3.4 hours (p = 0.0024), $N_4$ at 6.9 ± 1.7 hours (p<0.0001), SOH at 9.3 ± 3.8 hours (p = 0.0004) and $N_4$SOH at 10.4 ± 1.7 hours (p<0.0001; p values compared with sulfamethoxazole).

**Table II.** *In vitro* protein binding of sulfamethoxazole and its metabolites

| Compound | Concentration (mg/L) | Protein binding (%) | CV (%) |
|---|---|---|---|
| Sulfamethoxazole | 52.0 | 67.2 | 4.3 |
| 5-Hydroxysulfamethoxazole | 9.9 | 40.3 | 5.4 |
| Sulfamethoxazole-$N_1$-glucuronide | 10.1 | 26.7 | 1.1 |
| $N_4$-acetylsulfamethoxazole | 9.6 | 88.1 | 4.7 |
| $N_4$-acetyl-5-hydroxysulfamethoxazole | 11.6 | 69.5 | 15 |
| $N_4$-hydroxysulfamethoxazole | [a] | [a] | [a] |

a Not stable in plasma.

*Abbreviation:* CV = coefficient of variation.

**Table III.** Apparent and true renal clearance values of sulfamethoxazole and its metabolites

| Compound | $CL_{R,apparent}$ (ml/min) | Protein binding (%) | $CL_{R,true}$ (ml/min) |
|---|---|---|---|
| Sulfamethoxazole | 2.7 ± 0.9 | 67.2 | 8.3 |
| 5-Hydroxysulfamethoxazole | 96.1 ± 23.7 | 40.3 | 161 |
| Sulfamethoxazole-$N_1$-glucuronide | 176 ± 33 | 26.7 | 240 |
| $N_4$-acetylsulfamethoxazole | 35.2 ± 5.6 | 88.1 | 296 |
| $N_4$-acetyl-5-hydroxysulfamethoxazole | 51.2 ± 10.4 | 69.5 | 168 |
| $N_4$-hydroxysulfamethoxazole | a | a | a |

a Not stable in plasma.

*Abbreviation:* $CL_R$ = renal clearance.

The absorption half-life ($t_{½abs}$) of sulfamethoxazole was 0.12 ± 0.14 hours, which was much shorter than that calculated for the metabolites. The $t_{½abs}$ of $N_4$ (2.3 ± 0.9 hours) was identical (p = NS) to that of Sgluc (1.3 ± 1.4 hours). The same was observed for the formation of the hydroxy metabolites SOH (4.7 ± 5.0 hours) and $N_4$SOH (4.5 ± 2.4 hours; p = NS).

The plasma elimination $t_{½}$ varied between 9.6 and 11 hours (9.7 ± 1.3 hours) for the parent drug and metabolites. The $t_{½}$ was slightly higher for Sgluc (15.2 ± 5.3 hours; p = 0.0352). The elimination $t_{½}$ derived from the renal excretion rate-time curves for all compounds was identical to that of the $N_4$-acetyl metabolite.

The mean residence times (MRT) of the metabolites (20 to 25 hours) were higher than that of the parent drug (14.4 ± 2.2 hours; p = 0.0044). The intrinsic mean residence time ($MRT_i$), defined as $MRT_{metabolite}$-$MRT_{parent\ drug}$, for $N_4$ was 5.7 ± 1.9 hours (p = 0.0006), for Sgluc was 9.6 ± 7.6 hours (p = NS); for $N_4$SOH was 10.1 ± 3.0 hours (p = NS); and for SOH was 20.6 ± 14.2 hours (p = NS; p values compared with MRT-S).

The intrinsic $MRT_{ij}$ of $N_4$SOH, when it was assumed to be formed from $N_4$, was 3.9 ± 1.9 hours, which did not differ significantly from the $MRT_i$ of $N_4$-acetylsulfamethoxazole (5.7 ± 1.9 hours).

The *in vitro* protein binding of sulfamethoxazole is shown in table II. The protein binding of sulfamethoxazole (67.2%) increases when the compound is acetylated (88%); it decreases when it is oxidised at the 5-position (40%). Glucuronidation at the $N_1$-position reduces the protein binding to 20%.

## Renal Clearance

The main metabolite in urine was $N_4$ (43.5 ± 5.6%), followed by sulfamethoxazole (14.4 ± 3.4%) and the $N_1$-glucuronide (9.8 ± 2.6%). The percentages of $N_4$SOH (5.3 ± 1.0%) and SOH (3.0 ± 1.0%) did not differ significantly (p = NS). Only 2.1 ± 0.9% of the N-hydroxylamine metabolite ($N_4$OH) was excreted. No difference was found between the fast and slow acetylators of sulfadimidine concerning the percentage of metabolites of sulfamethoxazole.

The highest apparent renal clearance was achieved with Sgluc with 176 ± 33 ml/min. When corrected for protein binding, the true renal clearance was 240 ml/min. The renal clearance value of SOH was 96.1 ± 23.7 ml/min, which was significantly lower than that for the $N_1$-glucuronide (p<0.0001). The renal clearance value of $N_4$SOH was 51.2 ± 10.4 ml/min and for $N_4$ was 35.2 ± 5.6 ml/min; these values were not significantly different. When corrected for protein binding, the true renal clearance values of SOH and $N_4$SOH were similar (161 *vs* 168 ml/min). The true renal clearance of $N_4$ was similar to that of the $N_1$-glucuronide (296 *vs* 240 ml/min).

Sulfamethoxazole had the lowest apparent renal clearance of 2.7 ± 0.9 ml/min and a true renal clearance of 8.3 ml/min (table III).

## Discussion

Sulfamethoxazole is relatively well absorbed after oral administration. The percentage of the dose excreted in the urine as parent drug and metabolites varied between 70 and 90%.[11-14] The lag time ($t_{lag}$) gives the sequence of appearance in plasma and an indication regarding the dissolution rate and the absorption rate of the parent drug and the apparent rate of formation of the metabolites ($t_{lag\ metabolite} - t_{lag\ parent}$).

The $t_{max}$ is also a hybrid parameter, determined by the processes of absorption or formation and the intrinsic rate of elimination. Absorption of sulfamethoxazole is readily completed as the $t_{max}$ occurs at 1 hour; those of the primary conjugates ($N_4$-acetyl-, and $N_1$-glucuronide) occur at 5 to 7 hours. The oxidation to SOH and $N_4$SOH proceeded at a slower rate but for a longer period; $t_{max}$ was achieved at 10 hours.

The absorption half-life of the metabolites is a hybrid of the rates of formation and elimination. Like the $t_{max}$, this variable demonstrated that the rates of formation of $N_4$-acetylation and $N_1$-glucuronidation were similar ($p = 0.36$), and as fast as the oxidation at the 5-position ($p = 0.08$). The $C_{max}$ and $AUC_{0-\infty}$ values of $N_4$ were 10 times higher than those of Sgluc. With equal absorption/formation rates and equal rates of elimination by renal clearance, it may suggest that the maximum velocity of the capacity-limited process ($V_{max}$) or the enzyme concentration of the N-acetylase(s) is 10 times higher than that of the $N_1$-glucuronyltransferase(s).

The apparent elimination half-life of all metabolites was similar, as has been demonstrated many times previously for sulfamethoxazole and its acyl metabolite.[11-14] A slight variation in $t_{1/2}$ was observed between sulfamethoxazole and $N_4$, the $t_{1/2}$ of sulfamethoxazole was 9 hours and that of $N_4$ was 10 hours. This occurred when the urine pH of the subject varied between pH 6 and 8, and renal clearance of sulfamethoxazole contributes noticeably to the overall oral clearance. When the $t_{1/2}$ of sulfamethoxazole was 10 hours, it was identical to that of the $N_4$-acetyl metabolite. In the latter case the rate of acetylation of sulfamethoxazole governs the $t_{1/2}$ of both sulfamethoxazole and $N_4$ (fig. 4). These differences were observed within one subject, but did not reach statistical significance in the whole group of volunteers. The metabolites SOH and Sgluc demonstrated very small AUC values; the plasma concentrations were 5 to 10 times the quantitation limit. In these cases, the parallelism of the renal excretion rate-time profiles demonstrated the similarity of the half-lives.

When the apparent plasma elimination half-lives of the metabolites are identical to that of the parent drug, the total body clearances of the metabolites are higher and the intrinsic MRT values must be smaller than that of the parent drug, which indeed was the case (table I). Sulfamethoxazole is eliminated predominantly by metabolic conversion into the metabolites, which in turn are eliminated predominantly by renal excretion. The acetylator phenotype for sulfadimidine did not result in measurable differences in the percentage of the metabolite, nor in differences in the half-lives.[13,14,21] Thus, in the *in vivo* situation an acetylator phenotype for sulfamethoxazole cannot be distinguished.[24]

Sgluc and $N_4$ showed the highest true renal clearance values as the result of glomerular filtration and active tubular secretion. All metabolites were excreted by active tubular secretion, as their renal clearance values exceeded the glomerular filtration rate. Furthermore, urine pH and urine flow have little effect on the renal clearance, and the renal clearance of $N_4$ can be reduced by probenecid comedication.[12,13] The renal clearance of the metabolites equalled their total body clearance, indicating that they were not further metabolised. The renal clearance of Sgluc was twice that of the calculated total body clearance, which may indicate that this glucuronide is synthesised in part by the human kidney, as previously reported for the methoxysulfonamides.[16-18]

The electrophilic metabolite $N_4OH$, which may be held responsible for the occurrence of side effects,[5,25] was excreted for 2.1% of the dose in the urine. This means that only the free or unreacted $N_4OH$ was excreted and that the part causing side effects was not measured. When comparing the pharmacokinetics of sulfamethoxazole between healthy volunteers and HIV-positive patients, discrepancies in metabolism may reveal a relationship with the occurrence of side effects in HIV-positive patients. If the percentage of $N_4OH$ excreted in the urine of HIV patients is less than that in healthy volunteers, it must be guaranteed that this is not the result of alterations in the C-oxidation, $N_4$-acetylation and $N_1$-glucuronidation.[26-28] This work is now under investigation.

## Conclusion

In conclusion, sulfamethoxazole is well absorbed from the gastrointestinal tract and biotransformed to 5 metabolites. It is oxidised and conjugated by acetylation and glucuronidation. The pharmacokinetics of the $N_1$-glucuronide have been reported for the first time. The parent drug was eliminated mainly by metabolism, while the metabolites were eliminated mainly by renal excretion, resulting in comparable apparent half-lives.

## References


1. Cribb AE, Spielberg SP. Hepatic microsomal metabolism of sulfamethoxazole to the hydroxylamine. Drug Metab Dispos 1990; 18: 784-7
2. Cribb AE, Spielberg SP. Sulfamethoxazole is metabolized to the hydroxylamine in humans. Clin Pharmacol Ther 1992; 51: 522-6
3. Rieder J. Metabolism and technique for assay of trimethoprim and sulfamethoxazole. J Infect Dis 1972; 128 Suppl: 567-73
4. Sigel CW. Disposition and metabolism of trimethoprim, tetroxoprim, sulfamethoxazole and sulfadiazine. In: Hitchings GH, editor. Inhibition of folate metabolism and chemotherapy. The origins and uses of co-trimoxazole. Berlin: Springer Verlag, 1983: 163-84
5. Ven AJAM van der, Koopmans PP, Vree TB, et al. Adverse reactions to co-trimoxazole in HIV infection. Lancet 1991; 338: 431-3
6. Ueda M, Kuribayashi K. Studies on metabolism of drugs. V. On the new metabolite of sulfisomezole in man. Yakugaku Zasshi 1964; 84: 1104-7
7. Ueda M, Kakagawa Y, Murakami N. Studies on metabolism of drugs. VI. On the new metabolite of sulfisomezole in man. Yakugaku Zasshi 1967; 87: 451-4
8. Ueda M, Takegoshi I, Koizumi T. Studies on the metabolism of drugs. X. New metabolites of sulfisomezole in man. Chem Pharm Bull 1971; 19: 2041-5
9. Ueda M, Takegoshi I, Koizumi T. Studies on the metabolism of drugs. XIII. Quantitative separation of metabolites in human urine after oral administration of sulfisomezole and sulfaphenazole. Chem Pharm Bull 1972; 20: 2047-50
10. Woolley JL, Ragouzeous A, Brent DA, et al. Sulfonamide crystalluria: isolation and identification of sulfamethoxazole and four metabolites in urinary calculi. In: Nelson JD, Grassi C, editors. Current chemotherapy and infectious disease. Washington DC: The American Society for Microbiology, 1980: 552
11. Vree TB, Hekster YA, Baars AM, et al. Pharmacokinetics of sulphamethoxazole in man: effects of urinary pH and urine flow on metabolism and renal excretion of sulphamethoxazole and its metabolite $N_4$-acetylsulphamethoxazole. Clin Pharmacokinet 1978; 3: 319-29
12. Vree TB, Hekster YA, Damsma JE, et al. Pharmacokinetics of $N_1$-acetyl-, and $N_4$-acetylsulphamethoxazole in man. Clin Pharmacokinet 1979; 4: 310-9
13. Vree TB, Hekster YA. In: Schönfeld H, editor. Pharmacokinetics of sulfonamides revisited. Antibiotics and chemotherapy. Basel: Karger, 1985; 34: 1-200
14. Vree TB, Hekster YA. In: Schönfeld H, editor. Clinical pharmacokinetics of sulfonamides and their metabolites. Antibiotics and chemotherapy. Basel: Karger, 1987: 37: 1-200
15. Vree TB, van der Ven AJAM, Verwey-van Wissen CPWGM, et al. Isolation and identification and determination of hydroxy and glucuronide metabolites of sulfamethoxazole from human urine by means of preparative high performance liquid chromatography; J Chromatogr 1994; 658: 327-40
16. Vree TB, Beneken Kolmer EWJ, Martea M, et al. High-performance liquid chromatography of sulphadimethoxine and its $N_1$-glucuronide, $N_4$-acetyl and $N_4$-acetyl-$N_1$-glucuronide metabolites in human plasma and urine. J Chromatogr 1990; 526: 119-28
17. Vree TB, Beneken Kolmer EWJ, Martea M, et al. Pharmacokinetics, $N_1$-glucuronidation and $N_4$-acetylation of sulfadimethoxine in humans. Pharm Weekbl [Sci] 1990; 12: 71-5
18. Vree TB, Beneken Kolmer EWJ, Hekster YA, et al. Pharmacokinetics, $N_1$-glucuronidation and $N_4$-acetylation of sulfa-6-monomethoxine in humans. Drug Metab Dispos 1990; 18: 852-8
19. Vree TB, Beneken Kolmer EWJ, Hekster YA. Pharmacokinetics, $N_1$-glucuronidation and $N_4$-acetylation of sulfamethomidine in humans. Pharm Weekbl [Sci] 1991; 13: 198-216
20. Vree TB, Beneken Kolmer EWJ, Hekster YA. High pressure liquid chromatographic analysis and preliminary pharmacokinetics of sulfaphenazole and its $N_2$-glucuronide and $N_4$-acyl metabolites in plasma and urine of man. Pharm Weekbl [Sci] 1990; 12: 243-7
21. Vree TB, O'Reilly WJ, Hekster YA, et al. Determination of the acetylator phenotype and pharmacokinetics of some sulphonamides in man. Clin Pharmacokinet 1980; 5: 274-94
22. Proost JH, Meijer DKF. MW/Pharm, an integrated software package for drug dosage regimen calculation and their therapeutic drug monitoring. Comput Biol Med 1992; 22: 155-63
23. Anonymous. SAS User's Guide. Basics 1982 eds. SAS Institute, Cary, 1982
24. Cribb AE, Nakamura H, Grant DM, et al. Role of polymorphic and monomorphic human arylamine N-acetyltransferases in determining sulfamethoxazole metabolism. Biochem Pharmacol 1993; 45: 1277-82

25. Cribb AE, Miller M, Leeder JS, et al. Reactions of the nitroso and hydroxylamine metabolites of sulfamethoxazole with reduced glutathione. Implications for idiosyncratic toxicity. Drug Metab Dispos 1991; 19: 900-6
26. Lee BL, Delahunty T, Safrin S. The hydroxylamine of sulfamethoxazole and adverse reactions in patients with acquired immunodeficiency syndrome. Clin Pharmacol Ther 1994; 56: 184-9
27. Ven AJAM van der, Mantel MA, Vree TB, et al. Formation and elimination of sulphamethoxazole hydroxylamine after oral administration of sulphamethoxazole. Br J Clin Pharmacol 1994: 38: 147-50
28. Ven AJAM van der, Koopmans PP, Vree TB, et al. Drug intolerance in HIV disease. J Antimicrob Chemother 1994; 34: 1-5

Correspondence and reprints: Dr *T.B. Vree*, Department of Clinical Pharmacy, Academic Hospital Sint Radboud, Geert Grooteplein Zuid 8, 6525 GA Nijmegen, The Netherlands.